EVALUATION OF TYROSINE KINASE RECEPTOR (TrK) EXPRESSION IN FOLLICULAR AND PLEXIFORM TYPES OF AMELOBLASTOMA – AN IMMUNOHISTOCHEMICAL STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH VI

ORAL PATHOLOGY & MICROBIOLOGY

2016 – 2019

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"EVALUATION OF TYROSINE KINASE RECEPTOR (TrK) EXPRESSION IN FOLLICULAR AND PLEXIFORM TYPES OF AMELOBLASTOMA – AN IMMUNOHISTOCHEMICAL STUDY"** is a bonafide work done by **Dr.G.Jisha,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the speciality of **DEPARTMENT OF ORAL PATHOLOGY AND MICROBIOLOGY,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2016-2019.

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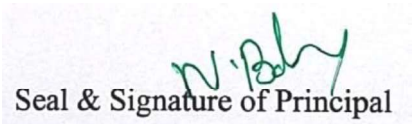

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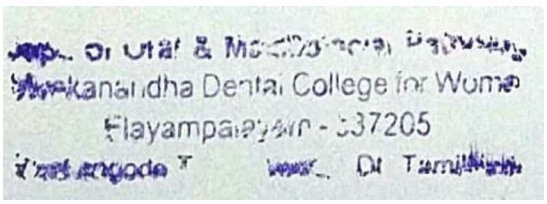

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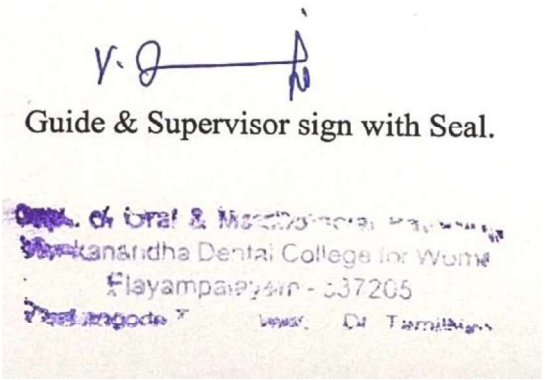

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I am indebted to my ancestors, my family and friends who paved the path before me upon whose shoulder I stand. – They are my strength.

With gratitude,

Dr. G. Jisha

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1.	**NTFs**	Neurotrophins
2.	**NGF**	Nerve Growth Factor
3.	**BDNF**	Brain-Derived Neurotrophic Factor
4.	**NT-3**	Neurotrophin-3
5.	**NT-4**	Neurotrophin-4
6.	**Trk**	Tyrosine Kinase
7.	**gp**	Glycoprotein
8.	**NF-kB**	Nuclear Factor Kappa B
9.	**MAPK**	Mitogen-Activated Protein Kinase
10.	**PDKs**	3-Phosphoinositide-Dependent Kinases

LIST OF ABBREVIATIONS

11.	**SH-PTP**	Src Homology Protein Tyrosine Phosphatase-2
12.	**SOS**	Son Of Sevenless
13.	**H&E**	Hematoxylin & Eosin
14.	**IHC**	Immuno Histo Chemistry
15.	**SP**	Signal Peptide
16.	**CC**	Cysteine Clusters
17.	**LRM**	Leucine-Rich Motif
18.	**Ig**	Immunoglobulin
19.	**TM**	Transmembrane Region
20.	**TK**	Tyrosine Kinase Catalytic Domain

LIST OF ABBREVIATIONS

21.	**BAD**	Bcl-2/Bcl-X-Associated Death Promoter
22.	**CREB**	CRE-Binding Protein
23.	**ERK**	Extracellular Signal Regulated Kinase
24.	**FRS**	Fibroblast Growth Factor Receptor Substrate
25.	**IRS**	Insulin Receptor Substrate
26.	**PI3K**	Phosphatidylinositol-3-OH Kinase
27.	**PKC**	Protein Kinase C
28.	**PLC**	Phospholipase C
29.	**RSK**	Ribosomal S6 Kinase
30.	**SH**	Src Homology

LIST OF ABBREVIATIONS

31.	**PBS**	Phosphate Buffered Saline
32.	**EDTA**	Ethylene Diamine Tetra Acetic acid
33.	**DPX**	Distrene Dibutylpthalate Xylene
34.	**DAB**	3, 3-Diaminobenzidene Tetra Hydrochloride
35.	**HRP**	Horse Radish Peroxidase
36.	**PLC-g**	Phospholipase C-G
37.	**EBV**	Epstein Barr Virus
38.	**CD**	Cluster Differentiation
39.	**GSK**	Glycogen Synthase Kinase
40.	**NK**	Natural Killer

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INTRODUCTION

INTRODUCTION

Ameloblastoma is an odontogenic neoplasm of the jaw. Ameloblastoma is a benign tumor in most ethnic groups and it the second common tumor of the jaw following odontoma. The preponderance of this tumor is seen around the third decade of life with equal gender predilection. Common site of occurrence is the mandibular posterior region followed by the maxillary posteriors. Ameloblastoma clinically presents as a slow growing swelling of the jaw without any associated pain, leading to facial deformity. On radiographic examination radiolucency is appreciated with thinning of cortices, root resorption of the associated teeth can also be appreciated. Origin of this tumor is thought to arise from odontogenic epithelium without ectomesenchyme and with presence of mature fibrous stroma. Microscopically, it resembles enamel organ of a developing tooth which lacks dental hard tissue formation. Being similar to enamel organ this tumor is aggressive. Ameloblastoma has two types of cells with different proliferative activity, these activities are based on their cytological pattern and histological variant. The peripheral ameloblast like cells are known for their anti-apoptotic character, while the central stellate reticulum like cells are known for their proapoptotic activity.[1-13]

Neurotrophins are proteins with specific role on non-neuronal and neuronal cells. Brain Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin-3 and Neurotrophin-4 are the four different neurotrophins. Low affinity receptors - gp75NTFR and High affinity receptors – TrK are present in family of neurotrophins. These neurotrophins bind to their corresponding receptors on the surface of responsive cells to carry out their biological effect. p75NTR binds to all proteins of family of neurotrophins. Activation of p75NTR, induces cell apoptosis and cell

survival by activating JNK pathway and NF-kB cell pathway respectively. Family of Trk consist of three genes namely TrkA, TrkB, TrkC. Trk receptors are activated by the neurotrophins and they mediate various activities. Activation of TrkA is done by NGF, while TrkB is activated by BDNF and NT-4/5, whereas TrkC is activated by NT-3. Trk receptors activation leads to cell differentiation, survival, proliferation, and apoptosis through PI3K/Akt pathway, Ras/MAPK pathway, and PLC- γ1 pathway.[14,16-21]

Immunoreactivity of TrK, NGF, p75, are studied in the various stages of odontogenesis. Varied immuno-expression was evident in pre-ameloblast cells during both presecretory and secretory stages. As cells of ameloblastoma resembles pre-ameloblast like cells at its periphery.[14,15] This study will evaluate the immunoreactivity of TrK receptor in follicular and plexiform types of ameloblastoma.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM

The study is aimed at evaluating the expression of Tyrosine Kinase receptor (TrK) in ameloblastoma in order to understand the possible role and mechanism of TrK.

OBJECTIVES

To analyze the Immunohistochemical expression pattern of TrK receptor in follicular type of ameloblastoma.

To analyze the Immunohistochemical expression pattern of TrK receptor in plexiform type of ameloblastoma.

To compare the Immunohistochemical expression pattern of TrK receptor among the follicular and plexiform types of ameloblastoma.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Tyrosine Kinase Receptor

Neurotrophins are proteins with specific role on non-neuronal and neuronal cells. Brain Derived Neurotrophic Factor, Nerve Growth Factor, Neurotrophin-3 and Neurotrophin-4 are the four different neurotrophins. Figure 1 represents Neurotrophins and their receptors. These neurotrophins bind to their corresponding receptors on the surface of responsive cells to carry out their biological effect. Low affinity receptors - gp75NTFR and High affinity receptors – TrK are present in family of neurotrophins. p75NTR binds to all proteins of family of neurotrophins. Family of Trk consist of three genes namely TrkA, TrkB, TrkC. Trk receptors are activated by the neurotrophins and they mediate various activities. Activation of TrkA is done by NGF, while TrkB is activated by BDNF and NT-4/5, whereas TrkC is activated by NT-3. Figure 2 represents a working model for receptor activation with p75 dominant and Trk dominant signaling cascade. Activation of p75NTR, induces cell apoptosis and cell survival by activating JNK pathway and NF-kB cell pathway respectively. Trk receptors activation leads to cell differentiation, survival, proliferation, and apoptosis through PI3K/Akt pathway, Ras/MAPK pathway, and Phospholipase C- γ1 pathway.[14,16-21]

Figure 1 - Neurotrophins and their receptors[17]

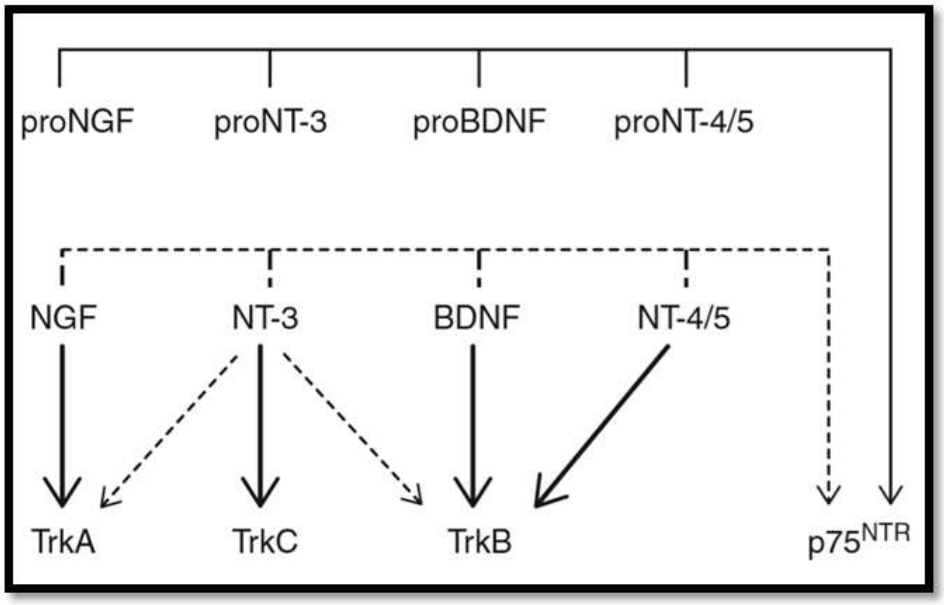
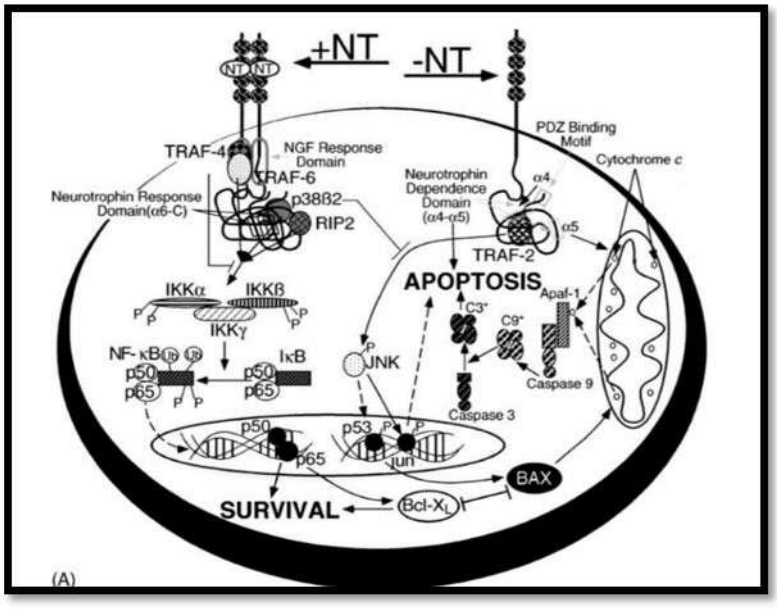
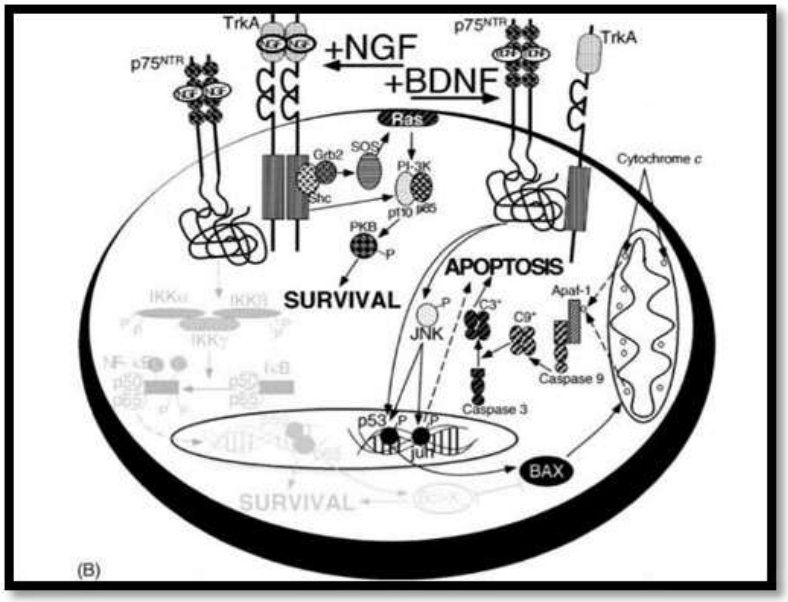


Figure 2 - A working model for receptor activation [18]

(A) p75-dominant system[18]



(B) Trk-dominant system[18]



Structure of Tyrosine Kinase Receptor

Three members of the TrK gene family are TrkA, TrkB and TrkC, which are identified till date. These genes encode for two distinct classes of receptors: Firstly, those with tyrosine-protein kinase activity, and secondly those non-catalytic receptors that share their respective extracellular and transmembrane sequences but that differ in their cytoplasmic region.

The role of the Trk tyrosine-kinase receptors is to mediate the trophic properties of the NGF family of neurotrophins, in neuronal and non-neuronal cells. The role of their noncatalytic isoforms are still unknown. Figure 3 and figure 4 represents structure and family of Trk receptor respectively.

Structural domains of these receptors include the following - Cysteine Clusters (CC), Immunoglobulin-like C2-type motifs (Ig), Leucine-Rich Motifs (LRMs), Transmembrane domain (TM), Juxta membrane region (JX), Signal Peptide (SP), and Tyrosine-Kinase catalytic domain (TK). A small open box in figure 3 indicates the six amino acid residues (VSFSPV) in neuronal specific TrkA receptor.

An open box located in the tyrosine- kinase domain of the TrkC K1 receptor indicates isoforms TrkC K14, TrkC K25, and TrkC K39 receptors. Sequences unique to the Trk noncatalytic receptor isoforms are presented in figure 3 by boxes of various shading are TrkB.Tl and TrkB.T2, as well as $TrkC^{TK-}158$, $TrkC^{TK-}143$, $TrkC^{TK-}113$, and $TrkC^{TK-}108$. The double boxes represent its derivation from different exons in $TrkC^{TK-}158$ and $TrkC^{TK-}143$ by these putative receptors.[19]

Figure 3 – Structure of Trk receptor.[20]

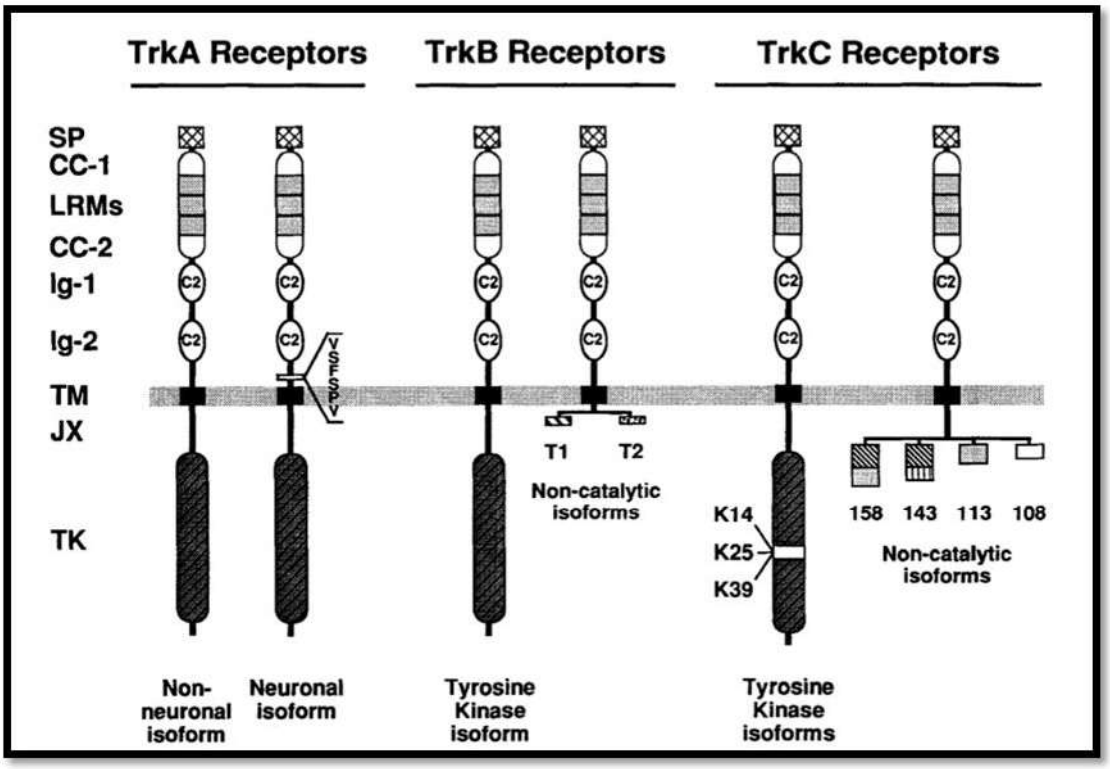


Figure 4 - The Trk Family of Neurotrophin Receptors[20]

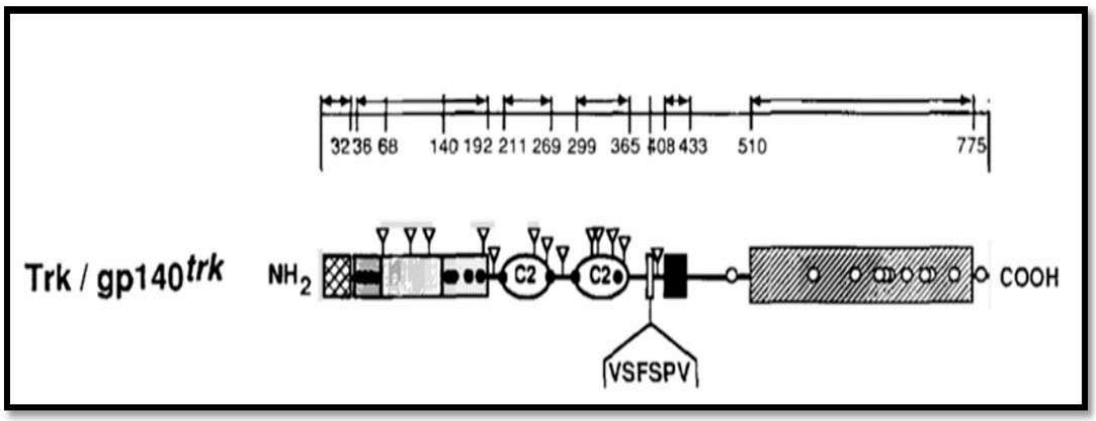
Gene	Receptor	Other Designations	Size	Structure
*trk*A	TrkA (nonneuronal)	gp140^{trkA}	790 aa[a]	Tyrosine kinase
	TrkA (neuronal)	gp140^{trkA}	796 aa	Tyrosine kinase
*trk*B	TrkB	TrkB^{TK+}/gp145^{trkB}	821 aa	Tyrosine kinase
	TrkB.T1	TrkB^{TK-}/gp95^{trkB}	476 aa	Noncatalytic
	TrkB.T2	TrkB^{TK-}	474 aa	Noncatalytic
*trk*C	TrkC	TrkC K1/TrkC^{TK+}/gp145^{trkC}	825 aa	Tyrosine kinase
	TrkC K14	TrkC K2/TrkC^{TK+}(14)	839 aa	Tyrosine kinase
	TrkC K25	TrkC K3/TrkC^{TK+}(25)	850 aa	Tyrosine kinase
	TrkC K39	TrkC K4/TrkC^{TK+}(39)	864 aa	Tyrosine kinase
	TrkC^{TK-} (158)		686 aa	Noncatalytic
	TrkC^{TK-} (143)		671 aa	Noncatalytic
	TrkC^{TK-} (113)		641 aa	Noncatalytic
	TrkC^{TK-} (108)		636 aa	Noncatalytic

[a] aa = amino acids.

TrkA Receptor

The first member of the Trk family is TrkA, it was discovered as an oncogene in which tropomyosin is fused to its kinase domain. The corresponding transmembrane protein was a protooncogene with structure of receptor tyrosine kinase. Two other additional putative members with interesting pattern of expression in the nervous system are present in this family.[21] Structural domains of TrkA receptor are represented in figure 5. TrkA receptor contains the following domains - Cysteine Clusters (CC) I and 11, The Signal Peptide (SP), Transmembrane region (TM), Leucine-Rich Motif (LRM), Immunoglobulin-like C2-type motifs (Ig) I and 11, and Tyrosine Kinase catalytic domain (TK). A small open box near the transmembrane domain indicates the six amino acid residues VSFSPV. Amino acid residue numbers flanking each of these motifs are indicated. The numbers in the residue indicates non-neuronal Trk receptors. Putative N-glycosylation sites are indicated by inverted triangles. Cys residues in the extracellular domain are represented by closed circles. Open circles indicate Tyr residues in the cytoplasmic domain.[19]

Figure 5 – Structural domains of TrkA receptor[19]

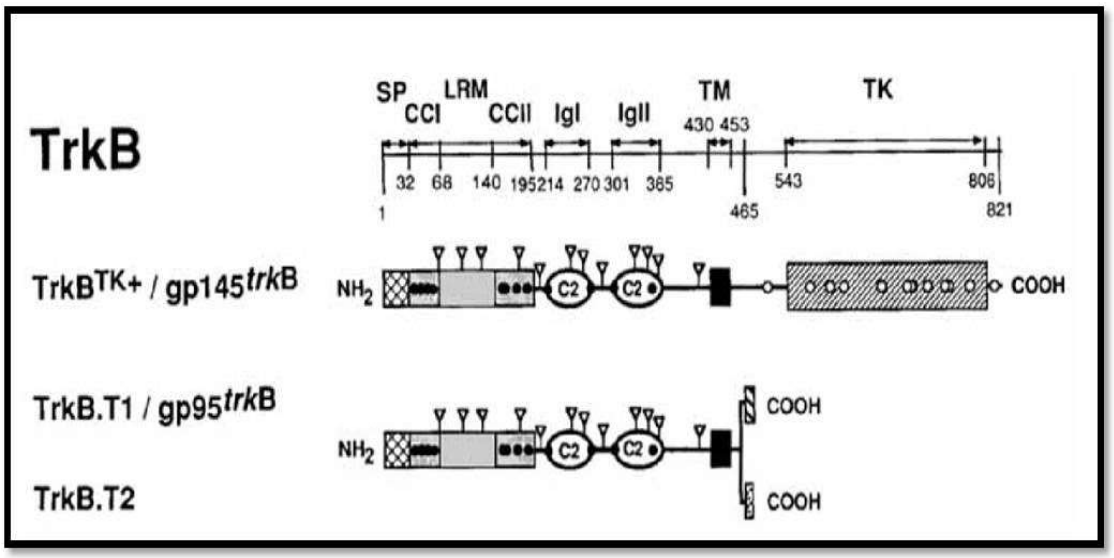


TrkB Receptor

The transcriptional pattern of TrkB locus is complex with at least eight different transcripts ranging in size from 0.7 to 9.0 kb and encodes at least two different classes of receptors.[22-24] Structural domains of the $TrkB^{TK+}$/$gp145^{trkB}$ tyrosine kinase receptors contains the following - Transmembrane region (TM), signal peptide (SP), leucine-rich motif (LRM), cysteine clusters (CC) I and 11, Tyrosine Kinase catalytic domain (TK) and Immunoglobulin-like C2-type motifs (Ig) I and 11. Figure 6 represents the structural domains of TrkB receptor. The noncatalytic $TrkB^{TK-}$ receptors are TrkB.Tl /$gp95^{trkB}$ and TrkB.T2. TrkB.Tl /$gp95^{trkB}$ receptor with its unique eleven amino acid residues are indicated by hatched boxes at their carboxy terminal. TrkB.T2 receptor with its unique nine amino acid residues are indicated by stippled boxes at their carboxy terminal.[19]

One of these receptors is $gp145^{trkB}$ which is designated as $TrkB^{TK+}$ contains all the canonical motifs of tyrosine kinase receptors which is about 821 molecules of amino acids and it is heavily glycosylated. The extracellular domains of the rat Trk and human $TrkB^{TK+}$ receptors are in homology which is about 57%. 38% identity is seen between them by the 12 Cys residues present in Trk. This homology is evenly distributed at the second and third LRMs and particularly within the second Ig-like domain.[25]

Figure 6 - Structural domains of TrkB receptor[19]

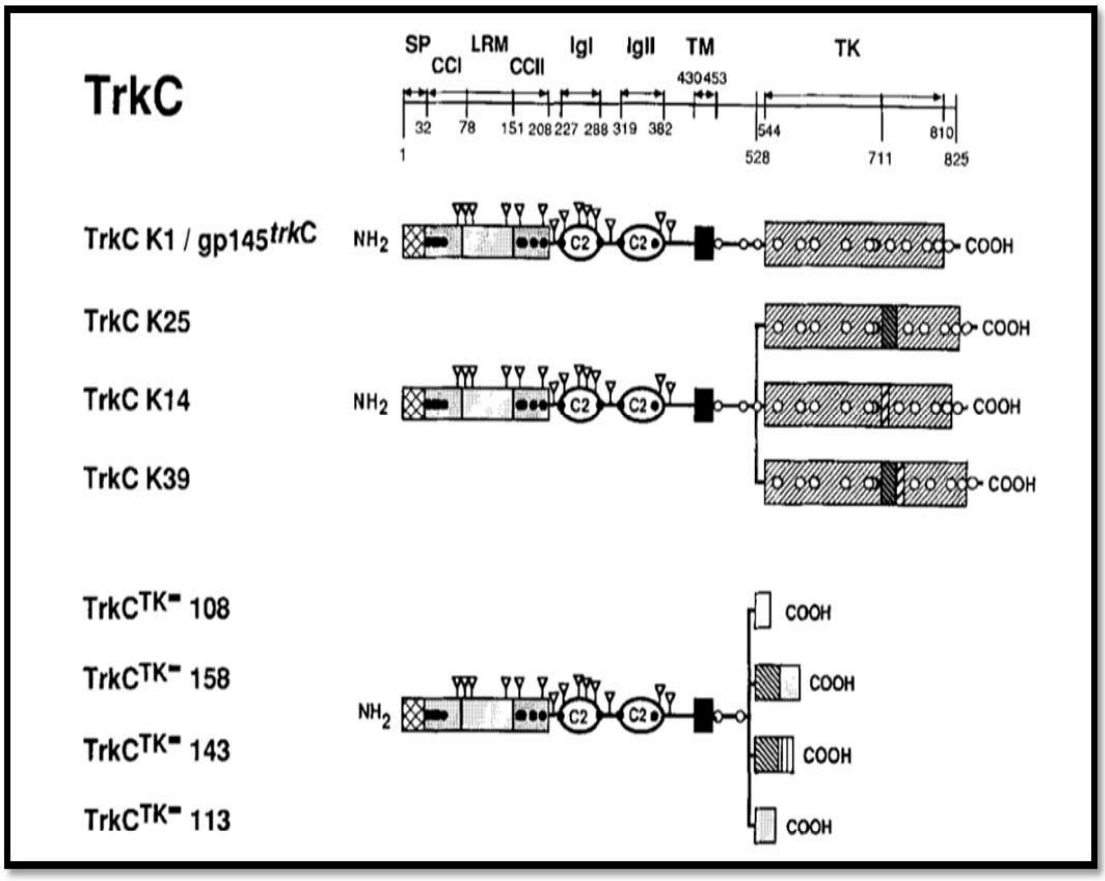


TrkC Receptor

Two classes of cell surface receptors encode for the TrkC locus. Till now there are, four isoforms of TrkC namely TrkC K25, TrkC KI /gp145^{trkC}, TrkC K39 and TrkC 14. The structural domains of TrkC contains the following - leucine-rich motif (LRM), the signal peptide (SP), Immunoglobulin-like C2-type motifs (Ig) I and 11, Cysteine Clusters (CC) I and 11, Tyrosine Kinase catalytic domain (TK) and Transmembrane region (TM). The product of a cDNA clone isolated from a porcine brain was first identified as TrkC K1. Figure 7 represents the Structural domain of TrkC receptor. The gray right-sided hatched box in the figure represents the isoforms TrkC K25 and TrkC K39 with additional 25 amino acid residues. A left-sided hatched box represents the TrkC K14 and TrkC K39 tyrosine kinase receptor isoforms with additional 14 amino acid residues. Sequences of four noncatalytic TrkC^{TK-} receptor isoforms are unique and are represented by boxes at their respective carboxy terminal ends. TrkC^{TK-}108 sequences derived from exon A are indicated by an open box. Sequences of TrkC^{TK-}158 and TrkC^{TK-}143, which are derived from exon B are represented by right-handed hatched box. Stippled boxes indicate TrkC^{TK-}158 and

$TrkC^{TK-}$113 derived from exon C. Vertically hatched box represents $TrkC^{TK-}$143 which are from exon D.[19]

Figure 7 – Structural domains of TrkC[19]



Trk-mediated signaling

Binding of Neurotrophin to the Trk receptors results in dimerization of receptor and activation of kinase. The cytoplasmic domain of each Trk receptor contains about 10 conserved tyrosine, the autoregulatory loop of the kinase domain are present in 3 conserved tyrosine. Further activation of the kinase is done by the Phosphorylation of these amino acids. Promotion of signaling by creating docking sites for adaptor proteins are done by the Phosphorylation of the other residues, which couples these receptors to intracellular signaling cascades, including the

Ras/extracellular signal regulated kinase (ERK) protein kinase pathway, phospholipase C-γ1 (PLC-γ1) and the phosphatidylinositol-3-OH kinase (PI3K)/Akt kinase pathway.[16,21,26]

In culture, Activation of Trk-kinase receptors by their cognate ligands initiates a cascade of signaling events which leads to either mitogenic stimuli for malignant transformation or neuronal differentiation. Trk receptors are activated by a two-step process, initially by the ligand- mediated oligomerization of receptor molecules at the cell surface, secondly by auto- phosphorylation of their tyrosine residues. Anchoring elements for downstream signaling are the phosphorylated tyrosine residues in the Trk-cytoplasmic domain. Enzymes and adapters are the two distinct classes of molecules to participate in this process. Both the enzymes and adapters activate Trk receptor by their interaction with the SH2 domains that recognize specific phosphor-tyrosine residues. With the binding of receptor, phosphorylation of enzymes takes place on tyrosine residues, and gets activated. Phosphorylation of Adapters, do not happen always. The role of these adapter molecules facilitates signaling by interaction of various molecules together on their cell membrane.[27,28]

Signaling through Ras

Neuronal differentiation and neuronal survival are regulated by Ras signaling either through the PI3K or the mitogen-activated protein kinase (MAPK)/ERK pathways. Proliferation or differentiation-inducing response depends on the transient or prolonged activation of the ERK pathway by the application of neurotrophins.[29]

Phosphorylation of Y490 initiates recruitment of an adaptor protein, in PC12 cells different adaptors initiate a cascade of signaling events depending upon the transient verses prolonged activation of ERK signal.[30]

Shc recruitment and its phosphorylation result in the recruitment of the Ras exchange factor Son of Seven less (SOS), and the membrane of a complex of the adaptor Grb-2 and thereby stimulating transient activation of Ras signaling. Activated Ras in turn activates the p38 MAPK/MAPK-activating protein kinase 2 pathways, PI3K, and the c-Raf/ERK pathway.[30]

Signaling through PI3K

PI3K signaling can be activated by Trk receptors, at least through two distinct pathways, the importance of these signaling differs among the various neuronal subpopulations. Ras-dependent activation of PI3K signaling pathway plays an important role through which neurotrophins promotes cell survival in many neurons.[31]

PI3K activation generates lipid products which in turn recruits many proteins containing pleckstrin-homology domains to the membrane, which include 3-phosphoinositide-dependent kinases (PDKs) and the Akt kinases. Activation of Akt is done by PDK at the membrane, after which AKT phosphorylates several important proteins for controlling cell survival.[32,33]

These include IκB, BAD (Bcl-2/Bcl-x-associated death promoter), the fork head transcription factor FKHRL1, human caspase-9 and glycogen synthase kinase (GSK) 3-β,.[34]

The transcription factor NF-κB is activated by the Phosphorylation of IκB. Recent studies have shown that the transcriptions activated by NF-κB, promotes neuronal survival.[35] The expression of apoptosis-promoting genes, such as FasL are controlled by the transcription factor FKHRL1.[34] The promotion of apoptosis in cultured neurons are seen with the Elevated GSK 3-β.[36]

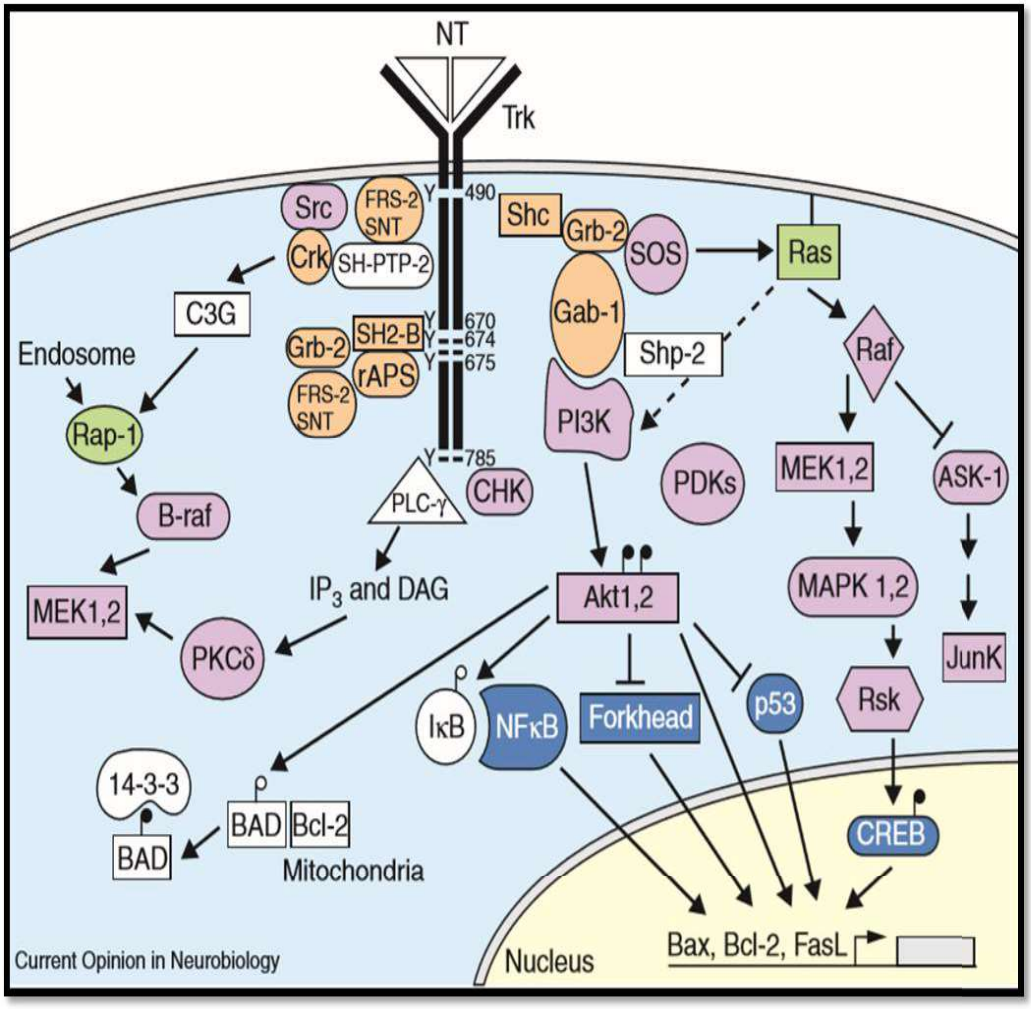
GSK 3-β activity increases with the withdrawal of neurotrophins and decreases with the phosphorylation by Akt. Control of apoptosis by many additional proteins also have their consensus sites for phosphorylation by Akt, but they do not have a direct substrate of this kinase.[32]

Signaling through PLC- γ1

TrkA and similar residues when placed on Trk receptor phosphorylates Y785 and recruits PLC-γ1. The phosphorylated and activated PLC-γ1, acts to hydrolyze phosphatidylinositides to generate inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). The generated IP3 induces the release of Ca2+ ions there by, increasing the levels of cytoplasmic Ca2+ ions, which in turn activates many pathways that are controlled by Ca2+ ions. Recent literatures have shown that NGF activates protein kinase C (PKC)-δ and a DAG-regulated protein kinase, which are required for neurite outgrowth and for the activation of ERK cascade.[37] Figure-8 represents the Neurotrophin signal transduction pathways mediated by Trk receptors.

Representations of Adapter proteins are colored orange, small G proteins green, kinases pink, and transcription factors blue. Abbrevations of CHK - Csk homologue kinase; APS - adaptor molecule containing PH and SH2 domains; SNT-suc-1-associated neurotrophic factor target; MEK - MAPK/ERK; P – serine /threonine (filled - phosphorylated).[21]

Figure 8 – Neurotrophin signal transduction pathways by Trk receptors. [21]



Signaling Pathway in Ameloblastoma

Hiroyuki Kumamoto and Kiyoshi Ooya, 2007- The Akt signaling pathway functions downstream of many growth factor receptors, similar to the Ras/MAPK pathway and provides survival signals that protect cells from apoptosis. PI3K activity was found to be required for the growth factor-dependent survival of a wide variety of cultured cell types. PI3K expression contributes to Akt activation in ameloblastoma tumours and is possibly involved in neoplastic changes of the odontogenic epithelium. The expression level of Akt in plexiform ameloblastomas was significantly higher

than that in follicular ameloblastomas shows the lower rate of tumour cell survival in follicular type.[38]

Laifa Hendarmin et al., 2008 - The activation of classical NFκB, a heterodimer containing p50 and p65 subunits, provides a survival signal in the majority of cell types. Studies found out that NFκB is closely associated with several diseases, such as autoimmune diseases and tumours. NFκB can directly activates pro-oncogenes such as cyclin D1 and c- Myc, and also has been implicated in the developmental and progression of human tumours. A study regarding the expression of NFκB in ameloblastoma suggests that the outer layer cells of ameloblastoma have higher anti-apoptotic activity in comparison to that of inner layer cells.[39]

Physiological Expression of Trk

1. Expression of TrkA has been seen on the surface of activated T- cells and in $CD8^+$ cells, $CD16/56^+$ NK cells as well as in $CD14^+$ monocytes.[40]
2. Expression of TrkB is seen in small population of $Cd4^+$ T-cells, limited surface expression on Th1 cells, sub population of CD^+8 cells, Epstein -Barr virus (EBV) transformed B- lymphocytes, surface expression on peripheral blood monocytes and in different macrophage population like the alveolar macrophages and in thymus derived macrophages.[40]
3. TrkC plays an important role in regulating the Th1/Th2 balance. Its expression is observed in $CD4^+$ lymphocytes, $CD8^+$ cytotoxic lymphocytes, $CD19^+$ B-cells and $CD16/56^+$NK cells.[40]
4. Trk receptors are expressed widely in non-neuronal and neural tissue, including the cortex, hippocampus, diencephalon, cerebellum, neurons of the peripheral nervous system, arteries, tooth buds, palate and the submaxillary gland.[19]

Pathological Expression of Trk

1. Marked expression of TrkB and TrkC plays an important role in epileptogenicity of dysplastic regions of the cerebral cortex.[41]
2. Immature inferior olivary neurons during the prenatal development express high level of TrkB and TrkC in the brainstem.[41]
3. Neuroblastoma, the most common extracranial solid cancer expresses TrkA, truncated TrkB lacking the tyrosine kinase domain, and TrkC.[41]
4. Cells of pheochromocytoma- a neural crest tumor Overexpresses TrkA.[41]
5. Co- localization of p75NTR and TrkA are seen on the sub population of cells in olfactory neuroblastoma, also known as esthesioneuroblastoma.[41]

6. Protection of hippocampal neurons from the effects of stroke, ischemia and excitotoxicity are mediated by the cooperative role of p75NTR in TrkA mediated signaling through the Akt-PI3 Kinase pathway.[41]
7. Expression of p75NTR, TrkA, TrkB and TrkC increased in bronchoalveolar eosinophils following allergen exposure.[41]
8. Neurotrophin- 3 and TrkC resist the endogenous and exogenous toxic brain injury and helps in regulation and maintenance of blood-brain barrier through the effects on the microvasculature.[41]
9. Proliferation promoting effect of TrkA is detected by its immunoreactivity in ovarian carcinoma in primary and metastatic sites.[42-45]
10. Advanced disease stage, high histological grade, and poor survival is correlated by the absence of TrkA and p75 expression in esophageal carcinoma.[46]
11. Medullary thyroid carcinoma progression is associated reduced expression of TrkB and elevated TrkA and TrkC expression.[47]

MATERIALS AND METHODS

MATERIAL AND METHODS

Study samples consist of 40 paraffin embedded tissue blocks, were selected from the archives of Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Tiruchengode. The study sample comprises of 20 follicular and 20 plexiform types of ameloblastoma. Two serial sections of 3-4.5 microns thickness of the study samples were sectioned. One section was stained using Haematoxylin and Eosin stain, this helped in knowing the histological pattern of ameloblastoma. When two or more histological patterns were present the predominant pattern was considered as a final diagnosis. The other sections of study samples were stained immunohistochemically using TrK A+B+C primary antibody. Immunohistochemical procedure standardisation were done using Nerve tissue as positive control. Immunohistochemical reactivity were evaluated depending upon the staining intensity as: Negative (-), Positive (+) and Strongly Positive (++)

Inclusion criteria

- Follicular and Plexiform histological variants of ameloblastoma are included.

Exclusion criteria

- Clinical types of ameloblastoma are not considered.
- Other histological varients of ameloblastoma other than follicular and plexiform.
- Recurrent ameloblastoma are not considered.

Equipment's and materials used in the study:

- LEICA RM 2125RT - Rotary microtome
- Slide warmer for deparaffinization
- Water bath
- Humidifying chamber
- Research microscope with photomicrography attachment
- PathnSitu Positively charged – hydrophobic microscopic slides
- Copelin jars
- Slide Holder
- Eppendorf tubes
- Micropipettes
- Plastic disposable pipette tips
- Cover slips
- Mayer's Hematoxylin
- Eosin
- Phosphate wash buffer - pH: 7.4
- 3% Hydrogen Peroxide
- Abcam - Rabbit Monoclonal Anti-TrkA+B+C antibody
- Thermo Scientific Secondary antibody kit
- DAB chromogen
- Distilled water
- Graded alcohol
- Xylene
- DPX mountant

Preparation of primary antibody and substrate chromogen:

- Abcam Rabbit Monoclonal Anti-TrkA+B+C primary antibody was diluted at a ratio of 1:250 in Phosphate Buffered Saline in a mini Eppendorf tube using micropipette.

- Substrate chromogen solution was prepared by mixing 1ml of buffered substrate solution in a calibrated Eppendorf tube with one drop (approx. 50µl) of DAB chromogen. The solution was freshly mixed prior to its application to tissue sections using pipette.

Preparation of Phosphate Buffer Saline preparation: pH 7.4

- Potassium dihydrogen phosphate – 2.4 gm
- Sodium chloride – 17 gm
- Di-sodium hydrogen orthophosphate – 17.5 gm
- Distilled water – 2 liters.

Preparation of Tris/EDTA buffer - Heat mediated antigen retrieval: pH 9.0

- Tris buffer granules – 6.05 gm
- Disodium EDTA – 0.75 gm
- Distilled water – 1 liter

METHODOLOGY

Hematoxylin and Eosin Staining:

- Formalin fixed tissue were routinely processed using graded alcohols, xylene and paraffin wax. Embedder tissues were cut into 3-5µm thickness sections and placed on egg albumin coated glass slides.

- Slides were kept on slide warmer for deparaffinization, dewaxed in xylene and hydrated through graded alcohol to water.

- Sections were stained with Mayer's hematoxylin for 3-5 minutes and bluing was done in running tap water for 10 minutes.

- Eosin staining was done for 2 minutes and these slides were dehydrated through graded alcohol, dried, cleared with xylene and mounted with DPX. Figure 9 represents Hematoxylin and Eosin staining kit.

Figure 9 - Hematoxylin and eosin staining kit

Immunohistochemical Staining:

Sectioning: Two to three serial sections of 3 to 4.5 µm thickness were made and taken on Positively charged – hydrophobic microscopic slides.

Deparaffinization: The sections were deparaffinized by heating on the slide warmer at 60°C for one and half hours. The sections were dewaxed in 2 changes of xylene, each of 15 minutes.

Rehydration: The sections were rehydrated in graded alcohol (100%, 80% and 50%), 5 minutes each and kept under water for 10 minutes.

Antigen Retrieval: The slides were placed in a coupling jar, with Tris EDTA buffer solution which in turn was kept in a pressure cooker containing water. The pressure cooker was then closed with the lid and brought to full pressure. Timing was noted down after the full pressure was reached and was kept for 10 minutes duration. The pressure cooker was allowed to cool down to room temperature before removal of slides.

IHC staining procedure: All the reagents stored in the refrigerator were brought to room temperature prior to immunostaining. All the incubations were performed at room temperature using a humidifying chamber. At no time the tissue sections were allowed to dry during the staining procedure.

Step 1: Blocking procedure: After tapping off the excess buffer from the slide, the sections were circled with a Pap pen after which it is covered with 3% hydrogen peroxide for 10 minutes, followed by it was treated with protein block for 10 minutes

to avoid cross reactions and to reduce non-specific binding which was then gently washed with PBS.

Step 2: Primary antibody application: Excess buffer was tapped off and the sections were covered completely with optimally diluted Rabbit Monoclonal Anti-TrkA+B+C antibody in 1:250 dilutions in PBS for one and half hours. Then the slides were washed gently with PBS and kept in the PBS buffer bath for 10 minutes. Figure 10 represents Abcam Rabbit Monoclonal Anti-TrkA+B+C antibody.

Step 3: Secondary antibody application: The slides were washed and treated with secondary antibody tagged with Poly Horseradish peroxidase enzyme (HRP) for 30 min. Figure 11 represents Thermo scientific secondary antibody kit.

Step 4: Substrate chromogen application: The slides were then washed with PBS and immunostaining was developed by treatment with freshly prepared DAB solution for 5 minutes following which it was washed in distilled water to remove excess chromogen.

Step 5: Counter stain: The slides were immersed in Mayer's Hematoxylin for 30 seconds, and bluing was done in running tap water for 10 minutes.

Step 6: Mounting: The sections were dehydrated in graded alcohol (50%, 80%, 100%), air dried for 10 minutes, cleared with xylene and mounted using DPX, a non-aqueous permanent mounting medium.

Figure 10 - Rabbit Monoclonal Anti-TrkA+B+C antibody (Abcam, Inc. USA)

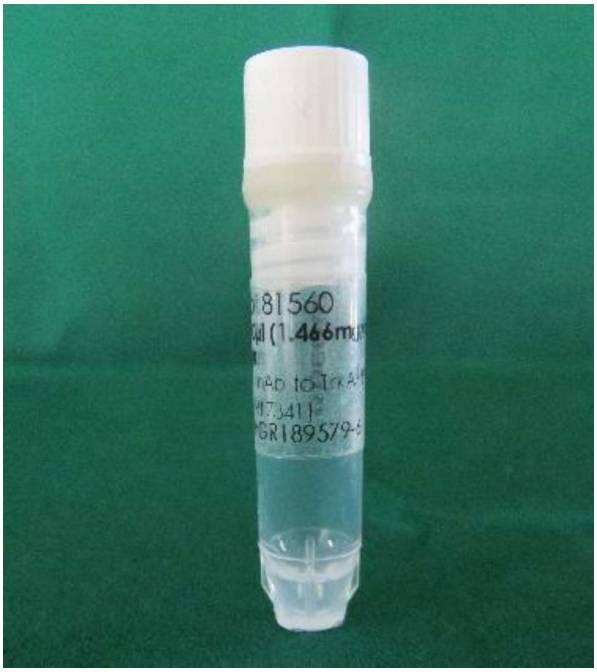


Figure 11 - Secondary antibody (Thermo scientific, UK)

Interpretation of staining:

- Presence of brown colored end product at the site of target antigen was considered as positive immunoreactivity.
- The immuno- stained slides were observed for positivity under 10x/40x magnifications and recorded with a high-quality photomicrograph.
- The staining was scored by evaluating the positive and negative immunoreactivity of each slides as: (-) Negative, (+) Positive, (++) Strongly positive.

Table 1 – Staining Interpretation

Types	Total no of Cases	Negative (-)	Positive (+)	Strongly positive (++)
Follicular	20			
Plexiform	20			

STATISTICAL ANALYSIS

Software used was Statistical package for social science SPSS version 16 (IBM CORP, Chicago, IL, USA). Statistical tests used are as follows:

1. For observer agreement: Cohen's kappa statistic was calculated
2. For intergroup comparison of the intensity of staining between follicular and plexiform ameloblastoma chi square test were used.
3. Frequency distribution of staining between plexiform and follicular pattern of ameloblastoma are separately represented for observer 1 and 2.
4. P value of < 0.05 was employed in all statistical comparisons.

RESULTS

RESULTS

ASSESSMENT OF TYROSINE KINASE RECEPTOR (TRK) EXPRESSION IN THE STUDY GROUP

Study samples consist of 40 paraffin embedded tissue blocks, were selected from the archives. The study sample comprises of 20 follicular and 20 plexiform types of ameloblastoma. Two serial sections of 3-4.5 microns thickness of the study samples were sectioned. One section was stained using Haematoxylin and Eosin stain, this helped in knowing the histological pattern of ameloblastoma. When two or more histological patterns were present the predominant pattern was considered as a final diagnosis. The other sections of study samples were stained immunohistochemically using TrK A+B+C primary antibody. Immunohistochemical procedure standardisation were done using Nerve tissue as positive control. Brown colour end product was considered as positive immunoreactivity under low and high-power magnification. Immunohistochemical reactivity were evaluated depending upon the staining intensity as: Negative (-), Positive (+) and Strongly Positive (++). The staining was scored by two observers evaluating the positive and negative immunoreactivity of each slide. The scores for strongly positive immunoreactivity were given as 2, for positive immunoreactivity as 1 and for negative immunoreactivity as 0.

DISTRIBUTION OF IMMUNOREACTIVITY AMONG THE PLEXIFORM AND FOLLICULAR TYPE OF AMELOBLASTOMA AMONG OBSERVERS

Two observers were employed to interpret the staining intensity of 20 samples of follicular and plexiform ameloblastoma each. The interpretation of both the observers are statistically evaluated using the SPSS version 16 software. The

distribution of staining intensity among follicular and plexiform ameloblastoma are mentioned by both observers are given as separate tables and bar diagrams. Interobservers variability was calculated using Kappa statistics and interpreted. Comparison between follicular and plexiform pattern of staining was calculated using chi square test for both the observer's interpretation and are represented in separate tables.

Immunoreactivity expressed among plexiform and follicular ameloblastoma noted by observer 1 is given in the Table 2. Among plexiform ameloblastoma 5 samples showed strongly positive immunoreactivity. Whereas 9 samples had positive immunoreactivity and 6 had negative reactivity. Thus, in plexiform ameloblastoma there was an overall 70% positive immunoreactivity and 30% negative immunoreactivity as noted by observer 1.

Among follicular 16 samples had Trk negativity which accounts for about 80% and only 4 samples which is about 20% had positive immunoreactivity. Number of samples with positive, negative and strongly positive reactivity as noted by observer 1 are represented in figure 12 as bar diagram.

On a whole 22 out of 40 samples as mentioned by observer 1 showed negative reactivity accounting for about 55%, whereas 13 samples showed positive reactivity which is about 32.5% and about 12.5% that is about 5 samples showed strong positivity. This overall frequency distribution as mentioned by observer 1 is represented in table 3.

Immunoreactivity expressed among plexiform and follicular ameloblastoma noted by observer 2 is given in the Table 4. Among plexiform ameloblastoma 3 samples showed strongly positive immunoreactivity which is about 15%. Whereas 11 samples which accounts for abut 55% had positive immunoreactivity and 6 samples which is about 30% had negative reactivity. Overall of 70% of samples in plexiform had positive immunoreactivity and the rest 30% had negative immunoreactivity. Among follicular 16 samples had Trk negativity which accounts for about 80% and only 4 samples accounting about 20% had positive immunoreactivity.

Number of samples with positive, negative and strongly positive reactivity as mentioned by observer 2 are represented in figure 13 as bar diagram. Over all 22 among 40 samples showed negative reactivity accounting about 55%, 15 samples showed positive reactivity which is about 37.5% and 3 samples which is about 7.5% showed strong positivity. This frequency distribution as mentioned by observer 2 is represented in table 5.

The overall expression of varying immunoreactivity among the 40 samples as stated by both the observers are represented in the table 6. This table shows that there was an agreement between negative immunoreactivity among observer's, while there was differences in staining interpretation among strongly positive and positive expressivity. Figure 14 represents the overall Number of samples with varying immunoreactivity as noted by observers.

Inter observer variability was calculated using Cohen's Kappa statistics which is represented in Table 7. A value of .912 was observed with an error of .059 and significance of .000. Interpretation of Kappa statistics are made by the value range given in table 8. From the interpretation table, the level of agreement is almost perfect in our study among the observers.

COMPARISON BETWEEN FOLLICULAR AND PLEXIFORM HISTOLOGICAL GROWTH PATTERN OF CENTRAL AMELOBLASTOMA

The expression of TrK was compared between the follicular ameloblastoma and plexiform ameloblastoma statistically by using 'Pearson's Chi Square' test. The chi square value for the immuno expression between follicular and plexiform ameloblastoma as noted by observer 1 is 11.469 with a p value of .003.

The chi square value for the immuno expression between follicular and plexiform ameloblastoma as mentioned by observer 2 is 10.812 with a p value of .004. The results were statistically significant between the two groups with a p value <0.005 as noted by both the observers.

The chi square value for both the observer 1 and observer 2 are represented in table 9 and table 10 respectively.

The H & E photomicrography of Nerve bundle, Follicular ameloblastoma and Plexiform ameloblastoma are represented by the figure 15, 16 and 17 respectively.

The photomicrography of TrK immuno expression in Nerve bundle, Follicular ameloblastoma and Plexiform ameloblastoma are represented by the figure 18, 19 and 20 respectively.

Table 2– Immuno reactivity expressed among Plexiform and Follicular Ameloblastoma by Observer 1

			Observer1			Total
			Negative	**Positive**	**Strongly positive**	
Ameloblastoma	Plexiform	Count	6	9	5	20
		% within ameloblastoma	30.0%	45.0%	25.0%	100.0 %
		% within observer1	27.3%	69.2%	100.0%	50.0%
	Follicular	Count	16	4	0	20
		% within ameloblastoma	80.0%	20.0%	.0%	100.0 %
		% within observer1	72.7%	30.8%	.0%	50.0%
Total		Count	22	13	5	40
		% within ameloblastoma	55.0%	32.5%	12.5%	100.0 %
		% within observer1	100.0%	100.0%	100.0%	100.0 %

Table 3 - Frequency of distribution of immunoreactivity among ameloblastoma by observer 1

		Frequency	Percent
Valid	Negative	22	55.0
	Positive	13	32.5
	Strongly positive	5	12.5
	Total	40	100.0

Table 4– Immuno reactivity expressed among plexiform and follicular ameloblastoma by Observer 2

			Observer2			
			Negative	Positive	Strongly positive	Total
Ameloblastoma	Plexiform	Count	6	11	3	20
		% within ameloblastoma	30.0%	55.0%	15.0%	100.0%
		% within observer2	27.3%	73.3%	100.0%	50.0%
	Follicular	Count	16	4	0	20
		% within ameloblastoma	80.0%	20.0%	.0%	100.0%
		% within observer2	72.7%	26.7%	.0%	50.0%
Total		Count	22	15	3	40
		% within ameloblastoma	55.0%	37.5%	7.5%	100.0%
		% within observer2	100.0%	100.0%	100.0%	100.0%

Table 5- Frequency of distribution of immunoreactivity among ameloblastoma by observer 2

		Frequency	Percent
Valid	Negative	22	55.0
	Positive	15	37.5
	Strongly positive	3	7.5
	Total	40	100.0

Table 6 - Crosstabulation of both observer1 and observer2

			observer2			
			Negative	Positive	Strongly positive	Total
Observer	Negative	Count1	22	0	0	22
		% within observer1	100.0%	.0%	.0%	100.0%
		% within observer2	100.0%	.0%	.0%	55.0%
	Positive	Count	0	13	0	13
		% within observer1	.0%	100.0%	.0%	100.0%
		% within observer2	.0%	86.7%	.0%	32.5%
	Strongly positive	Count	0	2	3	5
		% within observer1	.0%	40.0%	60.0%	100.0%
		% within observer2	.0%	13.3%	100.0%	12.5%
Total		Count	22	15	3	40
		% within observer1	55.0%	37.5%	7.5%	100.0%
		% within observer2	100.0%	100.0%	100.0%	100.0%

Table 7 – kappa statistics for inter observer variability

a. Not assuming the null hypothesis

	Value	Asymp.Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement Kappa	.912	.059	7.266	.000
N of Valid Cases	40			

b. Using the asymptotic standard error assuming the null hypothesis

Table 8 - Interpretation of Kappa Statistic:

Value of Kappa	**Level of Agreement**	**% of Data Reliable**
0-.20	None	0-4%
.21-.39	Minimal	4-15%
.40-.59	Weak	15-35%
.60-.79	Moderate	35-63%
.80-.90	Stong	64-81%
Above .90	Almost perfect	82-100%

Table 9 - Chi-Square Tests for observer 1

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.469[a]	2	.003
Likelihood Ratio	13.622	2	.001
Linear-by-Linear Association	11.094	1	.001
N of Valid Cases	40		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.50

Table 10 –Chi-Square Tests for observer 2

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.812[a]	2	.004
Likelihood Ratio	12.272	2	.002
Linear-by-Linear Association	10.315	1	.001
N of Valid Cases	40		

b. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.50

Figure 12 - Number of samples with varying immunoreactivity by observer 1

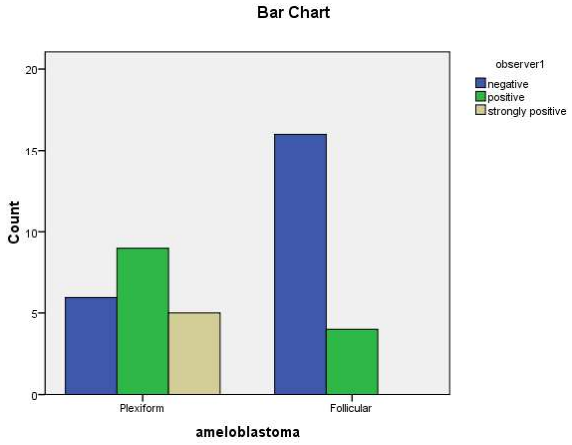


Figure 13- Number of samples with varying immunoreactivity as noted by observer 2

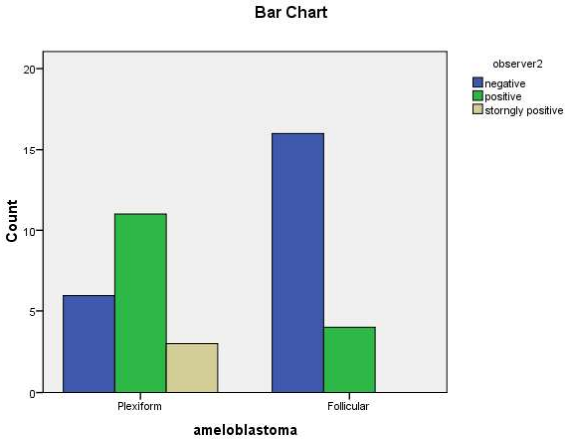


Figure 14 - Number of samples with varying immunoreactivity as noted by observers

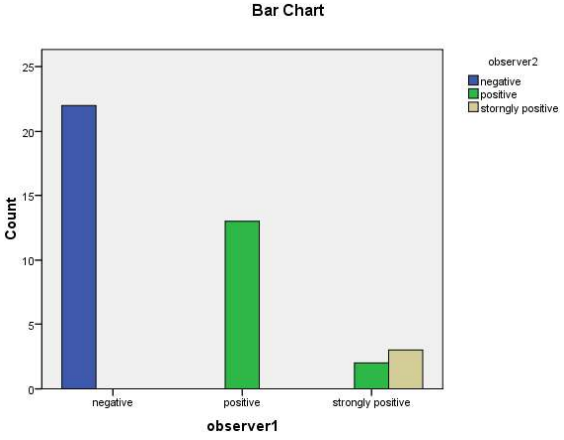


Figure 15 - H& E of nerve bundle (photomicrograph 20X)

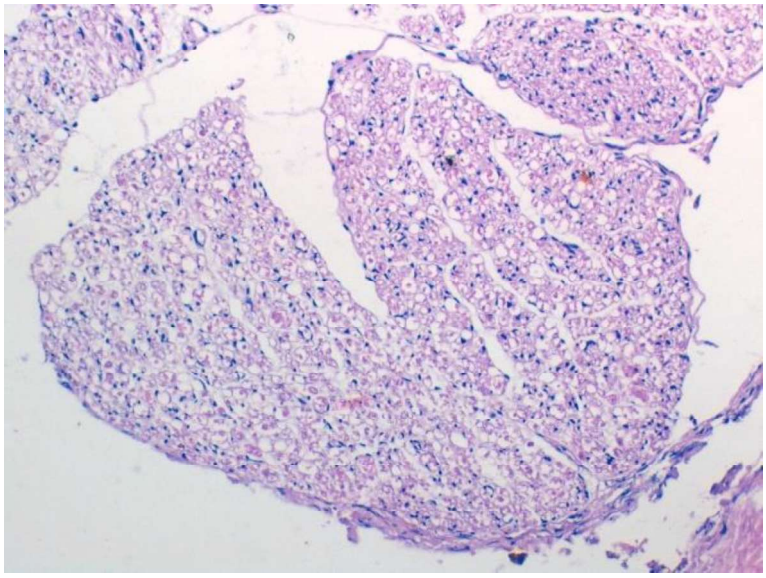


Figure 16 - H & E of follicular ameloblastoma showing follicles of odontogenic epithelium with tall columnar cells in the periphery and central stellate reticulum like cells (photomicrograph 40X)

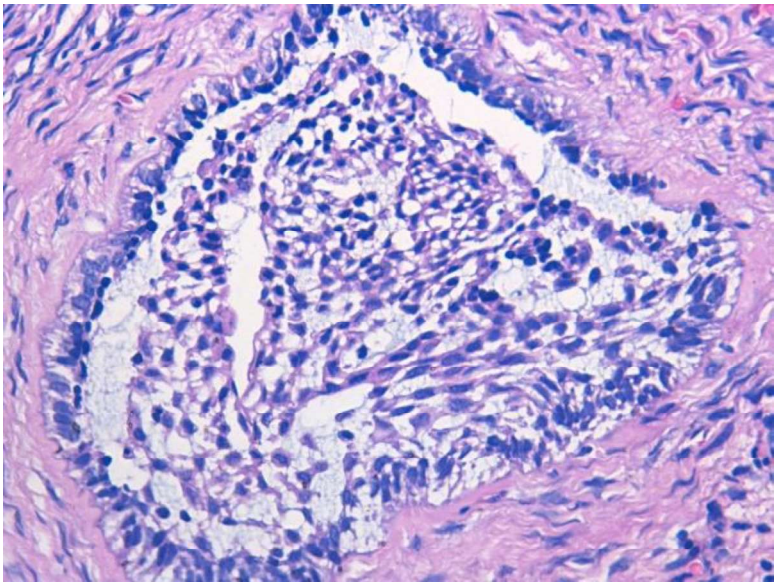


Figure 17 - H & E of plexiform ameloblastoma showing strands and cords of odontogenic epithelium (photomicrograph 40X)

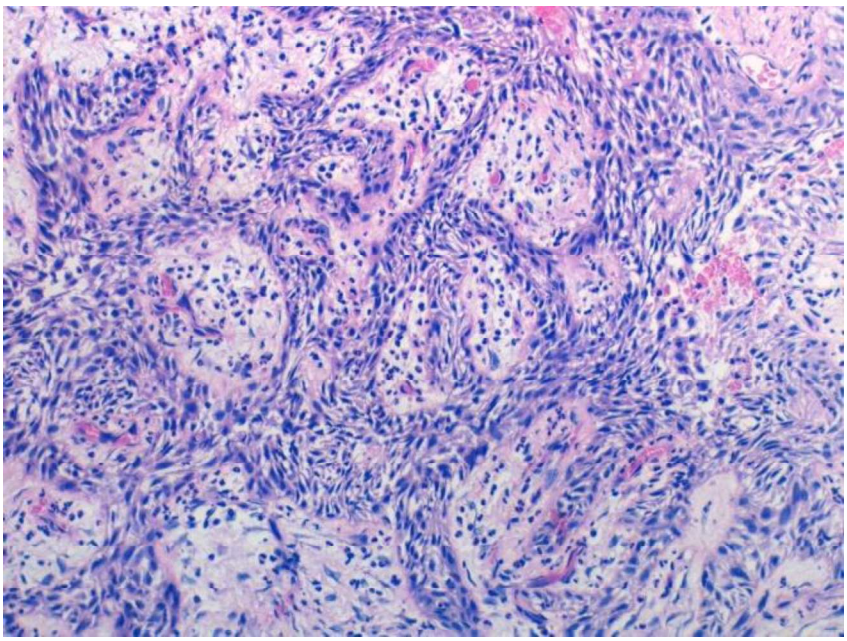


Figure 18 – Nerve bundle showing positive immuno-expression for TrK (photomicrograph 20X)

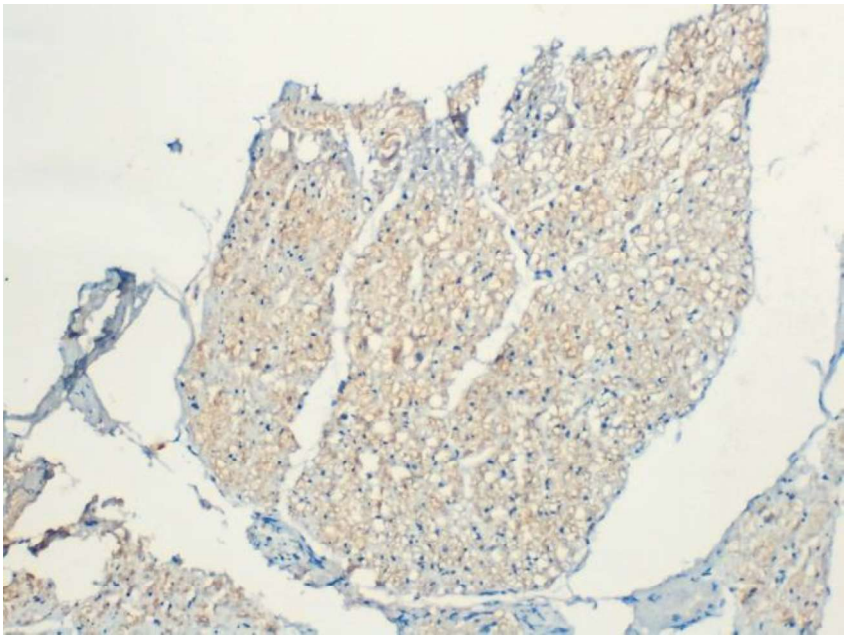


Figure19 - Follicular ameloblastoma showing TrK negative expression for TrK (photomicrograph 40X)

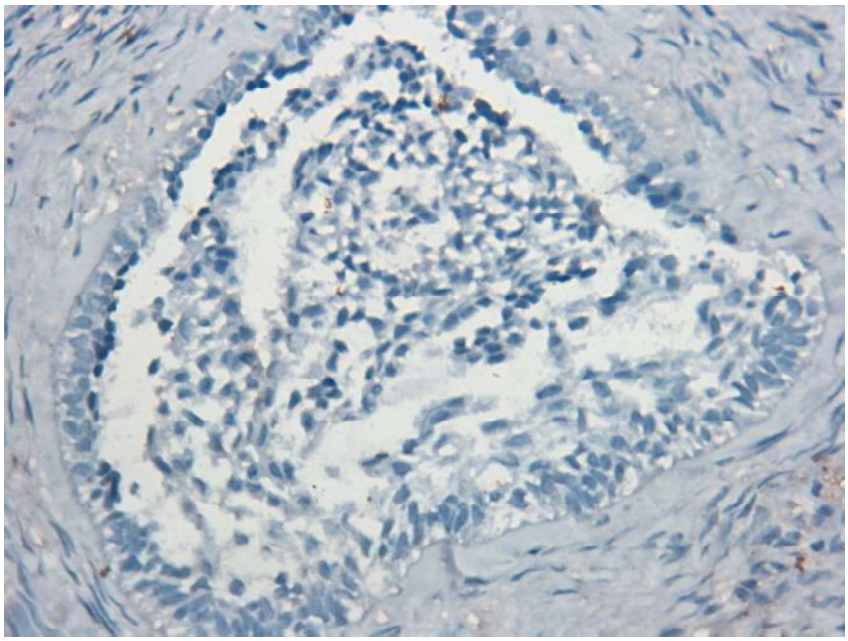
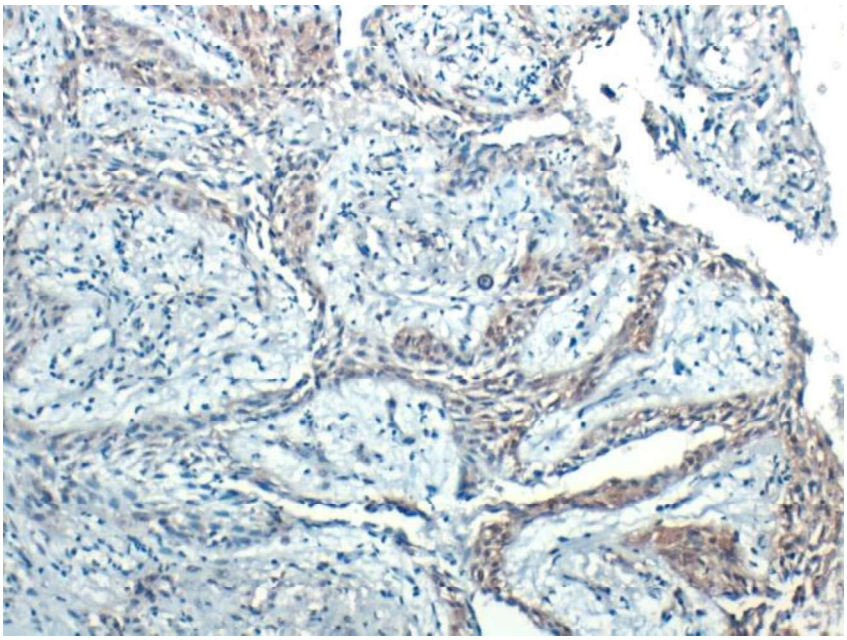


Figure 20 - Plexiform ameloblastoma showing TrK expression in the peripheral cells (photomicrograph 40X)



DISCUSSION

DISCUSSION

Robinson defined ameloblastoma as an "unicentric, non-functional, intermittent in growth, anatomically benign and clinically persistent" Ameloblastoma is an odontogenic neoplasm of the jaw, which is locally invasive. The mean age of occurrence is about 36 years, with posterior mandible being the prominent site of occurrence followed by the maxilla. Lesion appears as a painless slow growing tumor without any metastatic potential. This tumor growth by cellular proliferation and matrix degeneration results in craniofacial deformities. Histologically this tumor contains central stellate reticulum like cells which can undergo apoptosis and ameloblast like cells with proliferating potential at the periphery. Ameloblastoma can transform into ameloblastic carcinoma with rapid growth potential, cytological atypia and with metastasis. [4,6,8,10,48,49,50]

Earlier research in odontogenesis by various authors had noted the Temporospatial expression of various neurotrophins receptors. Neurotrophins with its High and low affinity receptors, mediate several pathways which play a role in the survival, differentiation and death, of non- neuronal and neuronal cells.[14,15]

Mitsiadis et al., observed that the Immunoreactivity for tyrosine kinase receptor (TrK) to be positive in pre-ameloblast cells in early and late bell stages of odontogenesis. Even differentiating ameloblasts had positive immuno reactivity in these stages of tooth development, but the staining intensity got decreased as the maturation of ameloblast was taking place.[15]

Christian et al., and Becktor et al., studied the immuno reactivity of p75-NTR in the developing tooth. They observed a positive immunoreactivity of p75-NTR along the entire inner enamel epithelium and in the dental follicles. With the gradually

deposition of matrix and with the differentiation of inner enamel epithelial cells the immunoreactivity for low affinity p75-NTR disappeared.[51,52]

Ragunathan et al., observed the expression of low affinity p75-NTR ameloblastoma. Two variants of ameloblastoma was observed for immunoreactivity in his study namely follicular and plexiform variants. Positive immunoreactivity was seen in peripheral ameloblast like cells of both the variants of ameloblastoma. Positivity was seen predominantly in follicular variant of ameloblastoma which constituted about 83.3%. Plexiform variant of ameloblastoma had relatively less positive immuno-expression of about 10% when compared to the follicular variant. In the present study expression of Trk was evaluated in 40 samples of ameloblastoma. Among them 15 samples had positive expression which is about 37.5% and 3 samples had strongly positive immunoreactivity accounting for 7.5% and the rest of the samples showed negative immunoreactivity. Predominantly plexiform ameloblastoma had positive immunoreactivity for Trk when compared to follicular ameloblastoma. 14 out of 20 cases of plexiform ameloblastoma had positive Trk immunoreactivity, whereas only 4 out of 20 follicular ameloblastoma had positive Trk expression. The positive Trk expression was predominantly seen in peripheral cells of the tumour cords and islands. Few central stellate-like cells had weak immunoreactivity for TrK. This was in contrast to the study result of p75NTR by Ragunathan et al., in follicular and plexiform ameloblastoma.[53]

Luo et al., observed the immuno-expression of Bcl-2 in ameloblastoma cases. The study sample contained 27 follicular variants and 12 plexiform type of ameloblastoma. Bcl-2 expression indicates the anti- apoptotic effects with its positive immuno-reactivity. Positive immuno reactivity was seen in the ameloblast like cells

present at the periphery of 13 samples of follicular ameloblastoma and 7 samples of plexiform ameloblastoma. An overall of 20 samples of ameloblastoma consisting of both the variants had positive immuno – reactivity in his study. Follicular variant had preferably increased expression when compared to plexiform type.[54]

Study done by Piattelli et al., studied the expression Proliferating Cell Nuclear Antigen (PCNA) in ameloblastoma in order to evaluate the Proliferative activity of ameloblastoma. His study comprised of 4 samples of follicular ameloblastoma and 5 samples of plexiform ameloblastoma. Nuclei of the peripheral ameloblast like cell had positive immuno-expression. Follicular ameloblastoma had a range of 25.90 - 39.50 PCNA positive nuclei. Higher proliferative range of 35.60 - 44.10 was observed in the peripheral ameloblast like nuclei of plexiform ameloblastoma.[55]

Kumamoto et al., has evaluated the immunoreactivity for pAkt and PI3K in his study using 18 cases of plexiform ameloblastoma and 22 cases of follicular ameloblastoma. This study result showed that there was a strong immunoreactivity in peripheral ameloblast like cells and weak reactivity in central stellate reticulum like cells, which was similar to the expression in our study. The level of immunoreactivity to PI3K was significantly higher in plexiform ameloblastoma than in follicular ameloblastoma. The increased expression of pAkt and PI3K suggest its role in tumourigenesis by activating the Akt signaling cascade.[38]

As stated by Meng et al., NF-κB activation provides a survival promoting signal. Thought it was believed that NF-κB and Akt signaling cascade act distinctly, they converge. The downstream signaling pathway of Nuclear Factor κB (NF-κB), is the Akt signaling pathway, which means regulation of such signal is due to the regulation of NF-κB.[56]

Guttridge et al., stated that transcriptional factor NF-κB controls apoptosis by expression of inducible target genes and mediate cell growth. NF-κB controls important functions like pRb hyperphosphorylation, activation of cyclin D1 expression and to promote G1-S progression in cell cycle.[57]

Hendarmin et al., in his study observed that 20 out of 24 ameloblastoma irrespective of their histological variant expresses anti-p65 NF-κB with varying immunoreactivity in both peripheral and central cells of ameloblastoma. Follicular variant had stronger immuno-expression of p65 NF-κB in the peripheral cells than the central stellate reticulum like cells.[39]

NF-κB activates distinct prosurvival bcl-2 family proteins which is a direct transcriptional target for NF-κB. Activation by NF-κB, suggests that these factors play a role in the inhibition of cell death by Rel/NF-κB differentially regulating the expression of Bcl-2 related death inhibitors and by directly activating the expression of Bcl-X_L as stated by Chen et al.[57,58]

Significant expression of high affinity Trk receptor in peripheral ameloblast like cells of plexiform ameloblastoma was appreciated in the present study, whereas the low affinity p75NTR expression was significant in the peripheral ameloblast like cell of follicular ameloblastoma as stated by Ragunathan et al.[53]

From these literatures, the cell survival property of ameloblastoma are predominantly contributed by the ameloblast like cells present at the periphery. These cells have anti apoptotic property and proliferative capacity. Molecular pathways in ameloblastoma growth and survival has been studied in recent years. The contributing role of Trk receptor in this entity is still not known. Trk receptor can mediate various intracellular signaling cascade via the PI3K/Akt kinase pathway, the Ras pathway,

and PLC-γ1 pathway. The association between p75NTR and Trk and its downstream molecules in ameloblastoma was not made through previous studies. This limits the present study in finding out the complete detail of Trk role in follicular and plexiform ameloblastoma.[4,10,14,16-21,26]

Further researches are needed to evaluate the role of individual Trk receptors namely TrkA, TrkB and TrkC on the variants of ameloblastoma in order to analyse the reason behind the differed biological behaviour among them and to account a detailed signaling cascade in this tumor.

SUMMARY &CONCLUSION

SUMMARY AND CONCLUSION

The possible role of TrK receptor immuno-expression in ameloblastoma was evaluated in the current study. This study was carried out in 40 paraffin embedded samples of ameloblastoma comprising equal number of follicular and plexiform histological variants of ameloblastoma.

4-micron thickness sections were obtained from the samples for IHC procedure. These sections were subjected to IHC procedure using TrK A+B+C as the primary antibody. Detection of brown colour was considered as positive immuno-expressivity. The immuno reactivity of these sections were interpreted by two observers.

Among 40 samples, 22 samples showed negative immuno reactivity, whereas 13 samples showed positive reactivity and about 5 samples showed strong positivity. Among observer's there was an agreement between negative immunoreactivity, while there were differences pertaining to strongly positive and positive immuno reactivity.

Cohen's Kappa statistics was calculated for inter observer variability. A kappa value of 0.912 was observed with almost perfect agreement among observers in the present study.

The chi square value for the immuno expression between follicular and plexiform ameloblastoma as noted by observers were 11.469 and 10.812 with a p value <0.005.

In the present study, Trk receptor showed varied expression in plexiform and follicular variants of ameloblastoma. The positive expression of TrK was found

predominantly in peripheral pre-ameloblasts like cells in most of the plexiform ameloblastoma and in few follicular ameloblastoma.

The positive expression of Trk in ameloblastoma helps in elucidation of the possible intracellular signal regulation mechanism in this tumor progression and proliferation. Thus, TrK could play a possible role of an activator in this tumour cell proliferation and tumour progression. The varied expression of Trk could be the possible reason behind the differed biological behaviour between follicular and plexiform ameloblastoma.

By obtaining clear knowledge on altered molecular signaling pathways in this neoplasia will definitely elucidate mechanisms of tumorigenesis, tumor differentiation, and tumor progression which may bring us to non-surgical approach for ameloblastoma treatment in near future. Further scientific researches and evidence are needed for the thorough knowledge about the relationship of tyrosine kinase receptor and the ameloblastoma tumor progression and proliferation. Further researches are needed to evaluate the role of individual Trk receptors namely TrkA, TrkB and TrkC on the variants of ameloblastoma in order to analyse the reason behind the differed biological behaviour among them and to account a detailed signaling cascade in this tumor.

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ANNEXURE

INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST

Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945.

Dr. J. Baby John	**Chair Person**	**Dr. (Capt.) S. Gokulanathan**	**Member Secretary**
Mr. K. Jayaraman	Social Scientist	Mr. A. Thirumoorthy	Legal Consultant
Dr. R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
Dr. B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Dr. Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/31/2015 Date: 05.11.2016

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. Jisha. G

Title: Evaluation of Tyrosine Kinase Receptor (TrK) expression in Follicular and Plexiform types of ameloblastoma - An Immunohistochemical study.

Institutional ethics committee thank you for your submission for approval of above proposal. It has been taken for discussion in the meeting held on 25.10.16.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College for Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
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DENTAL COLLEGE FOR WOMEN
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